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Higher reticulocyte counts are associated with higher mortality rates in hemodialysis patients: a retrospective single-center cohort study

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Abstract

Background: We assessed laboratory data related to mortality in hemodialysis (HD) patients. In our preliminary study, we examined all of the data for HD outpatients in our facility according to whether the patient had survived. A statistically significant difference was observed for the reticulocyte count, which has not previously been considered a prognostic factor. We subsequently verified the relationship between all-cause and cardiovascular mortality and reticulocyte count.

Methods: We retrospectively analyzed the data of 358 hemodialysis outpatients who were followed up for an average of 41.4 months. The patients were divided into quartiles according to the reticulocyte count levels.

Results: Higher reticulocyte counts were associated with female gender, an increase in interdialytic body weight gain, serum erythropoietin level, white blood cell count, and increased levels of lactate dehydrogenase, inorganic phosphorus, non-high-density lipoprotein, and glucose. As compared with patients in the lowest quartile, those in the highest quartile showed significantly higher adjusted hazard ratios (HRs) for all-cause (HR 3.12; 95% confidence interval (CI) 1.26 to 7.74) and cardiovascular (HR 4.93; 95% CI 1.24 to 19.56) mortality. For every 10^4 cells/μL increment in the reticulocyte count, the adjusted HRs for all-cause and cardiovascular mortality were 1.33 (95% CI 1.17 to 1.51) and 1.38 (95% CI 1.17 to 1.63), respectively. The association of reticulocyte count with all-cause and cardiovascular mortality was independent of other prognostic factors. Stepwise multivariable Cox analysis indicated that only age showed stronger association with all-cause mortality than reticulocyte count. Regarding cardiovascular mortality, reticulocyte count was found as the strongest progenitor. We also examined the relationship between the reticulocyte count and the temporal hemoglobin trend (a slope of changes in hemoglobin levels over time). A statistically significant negative correlation was found.

Conclusions: Higher reticulocyte counts were associated with higher mortality. We speculate that this result reflects tissue hypoxia, which results in a higher erythropoietin level, or a compensatory erythropoietic response due to the accelerated clearance of erythrocytes. Prospective studies are warranted to confirm our findings.

Keywords: Reticulocyte, Mortality, Hemodialysis, Anemia, Erythropoietin, Microvascular dysfunction

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Background

To improve the prognosis of chronic maintenance hemodialysis (HD) patients, it is important to identify simple and inexpensive prognostic factors. In our preliminary analysis, we examined data from a total of 1,814,698 HD outpatients. These data were obtained from January 4, 1999 to December 26, 2003. We divided patients into two groups depending on whether they had survived until the end of 2003. Student's t tests were performed for each comparison. In addition to the established prognostic factors, we found that the mean reticulocyte count was significantly higher in the non-survivor group. The means +/− standard deviations (SDs) of the reticulocyte count were 46.4×10^3 +/− 0.2×10^3 cells (sample count 8732) in the survivor group and 52.4×10^3 +/− 0.2×10^3 cells in the non-survivor group (sample count 1111) $p = 6.09$ E−15.

Reticulocytes are young red blood cells that develop from erythroblasts and circulate in the bloodstream for approximately 1–4 days before maturing into erythrocytes [1]. These cells provide a real-time assessment of the functional state of erythropoiesis [2] and are thus useful in both diagnosing anemias and monitoring the bone marrow response to therapy [3]. While reticulocyte count is widely measured in routine laboratory work, the clinical significance of the reticulocyte count in patients on chronic maintenance HD has yet to be clearly delineated [4].

In this study, we conducted a retrospective single-center analysis of the data of 358 patients under chronic maintenance HD to examine the association of reticulocyte counts with all-cause/cardiovascular mortality and hemoglobin trends (Hb trend).

Methods

Study design and patients

This is a retrospective observational study of 358 patients under regular maintenance hemodialysis at Hidaka Hospital. Patients were excluded from the study, when they had required hospitalization during 3 months prior to enrollment due to hematologic disorder, active bleeding, acute cardiovascular events, infectious diseases, or other comorbid conditions such as blood access troubles or traffic accidents. No patient had received chemotherapy for malignancy during the 3 months prior to enrollment. In addition, we did not include patients who had obvious inflammatory symptoms at the beginning of the enrollment period. The observation period was from January 2000 to July 2005, which was 5 years before darbepoetin therapy was introduced in our facility. The mean ± SD of the age of the cohort was 60.2 ± 12.6 years (range, 29 to 89 years), and the patients had been on maintenance hemodialysis for 8.5 ± 7.0 years, on average (range, 0.3 to 27.9 years). During the follow-up period, 52 patients were censored at departure to another dialysis unit. The mean follow-up period was 41.4 ± 16.6 months (range, 0.5 to 53.3 months).

The demographic and medical data were obtained from the medical records and interviews with the patients. The demographic data included age, gender, duration of dialysis, primary cause of renal failure, interdialysis weight gain, body mass index (BMI), dry weight, systolic blood pressure, diastolic blood pressure, vascular disease history, and smoking history (ever versus never). Vascular disease history was defined as a history of hospitalization due to coronary heart disease, heart failure, arrhythmia, valvular disease, aortic aneurysm, peripheral arterial disease, atrial septal defect, deep vein thrombosis, or cerebrovascular accident, including cerebral bleeding or infarction. The medication exposure data included the use of angiotensin-converting enzyme inhibitors (ACE-Is) or angiotensin receptor blockers (ARBs), the use of intravenous iron injections during the week prior to enrollment, and the average weekly dose of recombinant human erythropoietin (rHuEPO) at 1, 2, or 4 weeks prior to the observation period. None of the patients was receiving darbepoetin or continuous erythropoietin receptor activator (CERA) during the observation period in our cohort. Medical data were obtained from the records for the week prior to the enrollment. The doses of rHuEPO and iron were adjusted to maintain the hemoglobin level at approximately 10.0 g/dl, according to the dosage column of the Insurance Price List [5].

Blood samples were obtained on the first day of the week before the start of the dialysis sessions undertaken at 2-day intervals and were subjected to determination for Hb, reticulocyte count, white blood cell count (WBC), platelet count (Plt), transferrin saturation (TSAT), ferritin, albumin (Alb), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine (Cr), phosphorus inorganic (iP), total cholesterol, triglyceride, high-density-lipoprotein, blood sugar, C reactive protein (CRP), and erythropoietin (EPO). Determination for most of these parameters was undertaken as a part of the routine clinical work. The data obtained at a single time point within a week before enrollment were used for subsequent entire analysis. Concentrations of BUN were measured before and after a single dialysis session at enrollment to determine KT/V according to the procedure of Shinzato et al. [6]. The reticulocyte count was measured according to the method of Brecher [7].

Hb trend was obtained to determine the slope of the changes in the Hb levels over time, a best-fit (ordinary least squares) line of Hb over time until 2 weeks or 6 months after the start of the observation period was plotted for each subject; the temporal trend in Hb was represented by the slope of this line [8].

Statistical analyses

Data are expressed as mean ± SD. We used χ^2 or ANOVA to test for differences in the categorical or continuous factors, respectively, among the four quartiles of patients divided according to the reticulocyte count. The primary outcome was all-cause and cardiovascular mortality. Cardiovascular death was defined as death from coronary heart disease, heart failure, peripheral artery disease, cardiomyopathy, arrhythmia, cardiac arrest, valvular disease, infective endocarditis, rupture of aortic aneurysm, mesenteric artery occlusion, or cerebral bleeding. Survival curves were estimated by the Kaplan-Meier method, followed by a log-rank test. Univariate and multivariate analyses were conducted using the Cox proportional hazards model. Statistical significance was defined as $p < 0.05$. The models of association for all-cause mortality and death due to cardiovascular disease were built using a forward stepwise multivariate Cox modeling with $p < 0.05$ set as the inclusion criterion. The 20 candidate parameters were evaluated to identify associations between variables and outcome. The parameters tested were age, sex, diabetic nephropathy (DMN), duration of dialysis, interdialysis body weight gain, vascular disease history, use of ACE-I or ARB, systolic blood pressure, diastolic blood pressure, weekly dose of rHuEPO for a week before the enrollment (1w rHuEPO), Hb, WBC, Plt, iP, Cr, Alb, CRP, ferritin, TSAT, and reticulocyte count. The statistical contribution of each variable to the association of the outcome was assessed by the χ^2 statistic. Pearson correlations were used to test the relationships among the reticulocyte count, temporal trend of Hb, and dose of rHuEPO.

Results

Patient characteristics

We retrospectively analyzed the data of a total of 358 patients undergoing regular hemodialysis at Hidaka Hospital. The observation period extended from January 2000 to July 2005. During a mean follow-up of 41.4 months (0.5 to 53.3 months), 82 deaths were documented (heart failure $n = 19$, coronary heart disease $n = 14$, infection $n = 12$, malignant disease $n = 9$, peripheral artery disease $n = 3$, cardiomyopathy $n = 4$, gastrointestinal tract bleeding $n = 4$, cerebral bleeding $n = 3$, infective endocarditis $n = 2$, valvular disease $n = 2$, arrhythmia $n = 2$, cardiac arrest $n = 2$, rupture of aortic aneurysm $n = 1$, mesenteric artery occlusion $n = 1$, and miscellaneous $n = 4$), among which 53 were cardiovascular-related deaths.

Patients were divided into quartiles according to the reticulocyte counts. Table 1 shows the baseline characteristics of all the patients and of the patients in each reticulocyte count quartile. Higher reticulocyte counts were associated with female gender ($p = 0.01$), interdialysis body weight gain ($p = 0.02$), and a lower frequency of the use of ACE-I or ARB ($p < 0.01$). Meanwhile, age, duration of dialysis, blood pressure, history of smoking, weekly dose of rHuEPO, and use of iron injections were not correlated with the reticulocyte count.

As for the laboratory parameters, the serum Hb values were not significantly correlated with the reticulocyte counts. Patients with higher reticulocyte counts were more likely to have decreased TSAT, Hb trend (2 weeks and 6 months) and increase of such parameters as the serum EPO, WBC, LDH, BUN, iP, total cholesterol, triglyceride, non-high-density lipoprotein (non-HDL), and glucose.

Association of the reticulocyte count with all-cause and cardiovascular mortality

Crude survival was determined using Kaplan-Meier analysis with the log-rank test. The risk for all-cause death differed significantly among the reticulocyte count quartiles (log-rank test $\chi^2 = 28.34$, $p < 0.001$; Fig. 1a). In univariate Cox proportional hazard model, the all-cause mortality rate was highest in the highest quartile, and there was a survival gradient across quartiles (Table 2). After adjustments for background covariates, the hazard ratio (HR) for all-cause mortality in the highest versus the lowest quartile of reticulocyte count remained significantly high at 4.37 (95% confidence interval (CI) 1.94 to 9.84) (model 1 in Table 2). Additional adjustment for background and other prognostic factors Hb, WBC, Plt, TSAT, ferritin, iP, Cr, Alb, and CRP partially attenuated this estimate (HR 3.12; 95% CI 1.26 to 7.74), but the difference remained significant. The increase in mortality was in a relatively linear fashion. In the univariate analysis, the HR was significantly high for reticulocyte count increments of $1 \times 10^4/\mu L$ (HR 1.29; 95% CI 1.18 to 1.42; $p < 0.001$). The effect of reticulocyte count on the survival remained statistically significant after adjustments (model 1, HR 1.37, 95% CI 1.23 to 1.53; model 2, HR 1.33, 95% CI 1.17 to 1.51).

Significant increase of the cardiovascular death rates was also observed from the lowest to the highest reticulocyte count quartiles (log-rank test, $\chi^2 = 20.36$, $p < 0.001$) (Fig. 1b). The HR for cardiovascular death in the highest quartiles of reticulocyte count was significantly higher as compared with that in the lowest quartile at 5.67 (95% CI 1.94 to 16.59) (Table 2). The highest quartile of reticulocyte count, compared with the lowest, was significantly associated with the risk of cardiovascular death after adjustments (models 1 and 2). The effect of every 10^4 cells/μL increment in the reticulocyte count on the cardiovascular death also remained statistically significant after adjustments (models 1 and 2 in Table 2)

Table 1 Baseline patient characteristics

Characteristics		Reticulocyte count				*p*
	All Patients	Q1	Q2	Q3	Q4	ANOVA or χ^2
No. of patients	358	89	90	89	90	
Range of reticulocyte count (10^3/μL)	9.3 to 147.9	≤30.1	30.2 to 39.4	39.6 to 53.6	≤53.7	
Demographics						
Age (year)	60.2 (12.6)	60.4 (13.8)	60.4 (12.7)	61.0 (12.6)	59.1 (11.4)	0.78
Male gender (%)	67.3	78.7	70.0	64.0	56.7	0.01
Duration of dialysis (year)	8.5 (7.0)	7.9 (6.2)	8.8 (6.6)	8.8 (7.6)	8.7 (7.4)	0.80
Cause of renal failure						
Diabetes mellitus (%)	29.9	23.6	27.8	33.7	34.4	0.34
BMI	20.6 (3.0)	20.0 (2.4)	20.6 (3.0)	20.6 (2.8)	21.1 (3.5)	0.09
Dry weight (kg)	53.1 (10.3)	53.0 (9.0)	53.3 (12.1)	52.2 (9.1)	53.7 (10.7)	0.80
Interdialysis weight gain (%)	5.6 (1.9)	5.1 (1.9)	5.6 (1.9)	5.4 (2.0)	6.0 (1.7)	0.02
Smoking history (%)	44.9	48.8	42.9	45.3	42.5	0.84
Vascular disease history (%)	47.8	41.6	42.2	53.9	53.3	0.18
Medication						
ACE or ARB (%)	30.7	40.4	38.1	25.6	18.9	<0.01
Injection of Fe (%)	25.1	22.5	30.0	25.8	22.2	0.60
1w rHuEPO/week (IU/w)	3694 (3077)	3396 (2874)	4321 (2949)	3584 (3281)	3508 (3154)	0.19
2w rHuEPO/week (IU/w)	3727 (3079)	3459 (2906)	4415 (2971)	3576 (3255)	3496 (3079)	0.13
4w rHuEPO/week (IU/w)	3752 (3063)	3550 (2912)	4339 (2900)	3628 (3259)	3521 (3142)	0.25
Systolic blood pressure (mmHg)	151.9 (23.6)	154.8 (20.9)	155.5 (22.4)	146.8 (25.6)	150.33 (24.5)	0.05
Diastolic blood pressure (mmHg)	78.4 (11.6)	79.4 (12.1)	80.4 (11.1)	76.8 (10.0)	76.9 (13.0)	0.11
Laboratory parameters						
Reticulocyte count (10^3/μL)	43.4 (19.4)	22.5 (5.1)	35.1 (2.7)	45.8 (4.0)	69.6 (16.1)	<0.001
Hemoglobin (g/dL)	9.9 (1.1)	9.8 (1.2)	9.9 (1.0)	9.8 (1.0)	10.1 (1.2)	0.20
Mean corpuscular volume (fL)	97.8 (6.8)	97.2 (6.3)	98.9 (6.2)	97.9 (7.0)	97.0 (7.7)	0.22
White cell count (10^3/μL)	6.1 (2.1)	5.4 (1.5)	6.0 (1.8)	6.2 (2.2)	6.9 (2.5)	<0.001
Platelet count (10^3/μL)	185 (67)	172 (53)	182 (58)	187 (65)	199 (87)	0.06
Iron (μg/dL)	57.2 (23.3)	58.9 (25.9)	60.3 (25.0)	57.2 (20.0)	52.6 (21.4)	0.13
TSAT (%)	21.9 (10.1)	24.1 (11.8)	23.0 (10.1)	21.8 (9.1)	18.9 (8.5)	<0.01
Ferritin (ng/mL)	101 (135)	116 (167)	94 (107)	108 (160)	85 (92)	0.43
AST (U/L)	15.9 (8.8)	15.5 (6.4)	14.8 (6.0)	17.2 (12.6)	16.1 (8.5)	0.32
ALT (U/L)	14.2 (10.3)	13.6 (7.3)	13.7 (8.0)	15.4 (15.9)	14.0 (7.7)	0.62
Alkaline phosphatase (U/L)	251 (101)	253 (115)	249 (78)	246 (96)	255 (114)	0.94
Total bilirubin (mg/dL)	0.20 (0.09)	0.21 (0.10)	0.19 (0.06)	0.20 (0.10)	0.20 (0.09)	0.60
Lactate dehydrogenase (U/L)	335 (81)	323 (70)	322 (66)	342 (83)	353 (98)	0.03
Blood urea nitrogen (mg/dL)	77.4 (17.4)	70.1 (14.5)	80.3 (18.1)	79.1 (18.2)	80.1 (16.7)	<0.001
Creatinine (mg/dL)	11.7 (2.8)	11.5 (3.1)	12.0 (2.6)	11.5 (2.7)	11.8 (2.7)	0.63
Corrected calcium (mg/dL)	9.30 (0.88)	9.25 (0.84)	9.19 (0.92)	9.28 (0.88)	9.48 (0.87)	0.14
Phosphorus inorganic (mg/dL)	5.95 (1.50)	5.49 (1.42)	5.90 (1.33)	5.91 (1.56)	6.43 (1.53)	<0.001
Parathyroid hormone intact (pg/mL)	212 (191)	212 (206)	229 (172)	198 (292)	210 (184)	0.75
Albumin (g/dL)	3.72 (0.36)	3.71 (0.35)	3.80 (0.36)	3.68 (0.76)	3.68 (0.35)	0.07
Total protein (g/dL)	6.46 (0.51)	6.43 (0.49)	6.49 (0.50)	6.43 (0.58)	6.49 (0.50)	0.71
Glucose (mg/dL)	135 (62)	122 (40)	128 (47)	141 (72)	149 (78)	0.01

Table 1 Baseline patient characteristics *(Continued)*

Total cholesterol (mg/dL)	157 (34)	147 (31)	154 (33)	162 (31)	165 (36)	<0.01
High-density lipoprotein (mg/dL)	45 (14)	43 (12)	46 (14)	46 (15)	45 (15)	0.43
Triglyceride (mg/dL)	117 (68)	97 (48)	107 (62)	120 (62)	143 (87)	<0.001
Non-HDL (mg/dL)	112.1 (32.3)	104.2 (29.8)	108.1 (31.1)	116.4 (31.3)	119.9 (34.9)	<0.01
C-reactive protein (mg/dL)	0.57 (1.33)	0.41 (0.76)	0.45 (1.16)	0.56 (1.23)	0.86 (1.88)	0.11
Serum erythropoietin (U/L)	16.2 (22.8)	11.0 (9.0)	12.0 (8.0)	14.7 (12.0)	26.6 (40.4)	<0.001
Kt/V	1.23 (0.25)	1.19 (0.24)	1.26 (0.27)	1.24 (0.23)	1.23 (0.24)	0.41
Hb trend 0.5 mo (g/dL/week) $n = 353$	−0.02 (0.39)	0.01 (0.06)	0.08 (0.37)	−0.03 (0.31)	−0.12 (0.44)	0.01
Hb trend 6 mo (g/dL/week) $n = 338$	0.01 (0.07)	0.03 (0.06)	0.01 (0.06)	0.00 (0.05)	−0.01 (0.09)	<0.01

Results are displayed as mean (standard deviation). Patients were quartiled according to reticulocyte count. Q1, $\leq 30.1 \times 10^3$ cells/μL; Q2, 30.2 to 39.4×10^3 cells/μL; Q3, 39.6 to 53.6×10^3 cells/μL; and Q4, $\geq 53.7 \times 10^3$ cells/μL

Note: *BMI* body mass index, *ACE* angiotensin-converting enzyme, *ARB* angiotensin receptor blocker, *rHuEPO* recombinant human erythropoietin, *1w rHuEPO/week* the dose of rHuEPO per week for 1 week prior to enrollment, *2w rHuEPO/week* the average dose of rHuEPO per week for 2 weeks prior to enrollment, *4w rHuEPO/week* the average dose of rHuEPO per week for 4 weeks prior to enrollment, *TSAT* transferrin saturation, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *non-HDL* non-high-density lipoprotein, *Hb trend 0.5 mo* hemoglobin trend untill 0.5 month, *Hb tred 6 mo* hemoglobin trend untill 6 months

Table 3 shows the association models for all-cause and cardiovascular mortality. For each outcome, Cox proportional hazards models were built using a forward stepwise procedure. The variables are listed in order of their statistical strength of association with outcome, as represented by the χ^2 statistic. Reticulocyte count showed the second most powerful association with death from any cause, with χ^2 of 29.85. In this model, increased reticulocyte count showed among the most significant overall correlations of outcome, showing stronger statistical association than presence/absence of DMN, CRP, iP, and vascular disease history. Only age showed stronger independent association with outcomes. In our model for cardiovascular death, reticulocyte count showed the strongest association ($\chi^2 = 25.38$).

Correlation between the reticulocyte count and temporal trend of Hb

We next examined whether higher reticulocyte counts translated into a rise of the Hb levels during the following period. To determine the slope of the change in the Hb levels over time, we evaluated Hb trend until 2 weeks and 6 months after the start of the observation period. Among the 358 subjects, 8 died within the first 6 months of enrollment, before the trend in Hb levels could be determined. Hb concentrations were distributed in a very narrow range; the mean Hb trend was −0.02 +/− 0.39 g/dL/week at 2 weeks and 0.01 +/− 0.07 g/dL/week at 6 months. As shown in Fig. 2a, b, the reticulocyte count was negatively correlated with the temporal trend of Hb at 2 weeks ($r = -0.20$, $p < 0.001$, $n = 358$) as well as 6 months ($r = -0.21$, $p < 0.001$, $n = 338$).

Dose of rHuEPO and mortality

We examined all subjects in the cohort, including those who had received ($n = 281$) and those who had not received ($n = 73$) 1 week of treatment with rHuEPO (four patients' data were not available). We re-examined the prognostic impact of the reticulocyte count by separately analyzing subgroups of patients according to whether they had received rHuEPO. The prognostic impact of a high reticulocyte count with respect to the all-cause mortality remained significant not only in patients who had been treated with rHuEPO (HR per 10^4 cells/μL 1.33; 95% CI 1.20 to 1.47) but also in those who had not been treated with rHuEPO (HR per 10^4 cells/μL 1.28; 95% CI 1.03 to 1.59). Furthermore, the HR for all-cause mortality in the subgroup that had been treated with rHuEPO was not attenuated by an adjustment for the rHuEPO dose (HR 1.34; 95% CI 1.20 to 1.48). These data suggested that the association of the reticulocyte count with mortality was not dependent on the dose of rHuEPO.

Reproducibility of the association between reticulocyte count and all-cause and cardiovascular mortality

We also evaluated the association between the reticulocyte count and mortality at different time points. A total of 203 patients whose reticulocyte count data were available at 1 year prior to enrollment were divided into quartiles according to the reticulocyte count. A total of 32 deaths were documented, among which 25 were due to a cardiovascular cause. The range of each group was as follows: pQ1 $\leq 31.5 \times 10^3$ cells/μL, pQ2 (31.5×10^{33} cells/μL to 42.1×10^3 cells/μL), pQ3 (42.2×10^3 cells/μL to 54.2×10^3 cells/μL), and pQ4 $\geq 54.4 \times 10^3$ cells/μL. Survival curves were estimated via the Kaplan-Meier method, followed by the log-rank test. The risk for all-cause and cardiovascular death differed significantly among the reticulocyte count quartiles (all-cause death log-rank test $\chi^2 = 9.479$, $p = 0.02$; Fig. 3a/cardiovascular death log-rank test $\chi^2 = 9.914$, $p = 0.02$; Fig. 3b). In the

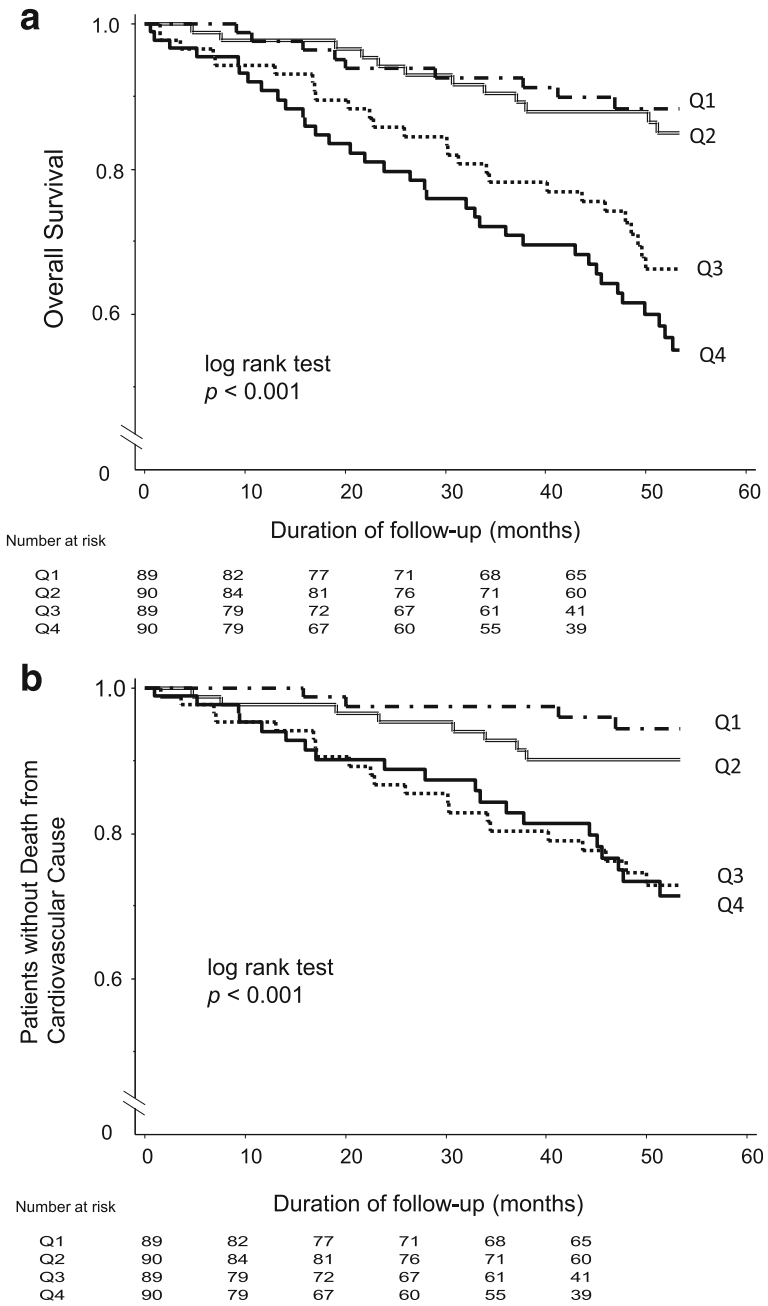

Fig. 1 Kaplan-Meier survival curves according to reticulocyte count quartiles. Patients were divided into quartiles according to the reticulocyte count. Q1, ≤30.1×10^3 cells/μL; Q2, 30.2 to 39.4×10^3 cells/μL; Q3, 39.6 to 53.6×10^3 cells/μL; and Q4, ≥53.7×10^3 cells/μL. **a** Overall survival; $p < 0.001$ as determined by a log-rank test conducted to compare the four groups. **b** Patients who had not died from cardiovascular causes; $p < 0.001$ according to a log-rank test conducted to compare the four groups

univariate Cox proportional hazard model, the HR was significantly higher for reticulocyte count increments of 1×10^4/μL for both all-cause mortality (HR 1.35; 95% CI 1.14 to 1.60; $p < 0.001$) and cardiovascular mortality (HR 1.41; 95% I 1.14 to 1.73; $p < 0.001$). Thus, our findings revealed that the reticulocyte count was related to mortality at 1 year prior to enrollment.

Discussion

One striking finding of this retrospective study was that the reticulocyte count showed very strong correlation with the all-cause and cardiovascular mortality in patients on maintenance HD. Multivariate analysis revealed that reticulocyte count was associated with all-cause and cardiovascular mortality, even after adjusting

Table 2 Adjusted association between quartile of reticulocyte count and mortality

	Unadjusted		Model 1		Model 2	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
All-cause mortality						
Q1	1 (Reference)	<0.001†	1 (Reference)	<0.001†	1 (Reference)	0.003†
Q2	1.23 (0.52 to 2.93)		0.95 (0.37 to 2.49)		0.71 (0.26 to 1.96)	
Q3	3.06 (1.43 to 6.52)		2.24 (0.98 to 5.16)		1.94 (0.83 to 4.58)	
Q4	4.37 (2.10 to 9.10)		4.37 (1.94 to 9.84)		3.12 (1.26 to 7.74)	
per 10^4 cells/μL	1.29 (1.18 to 1.42)	<0.001	1.37 (1.23 to 1.53)	<0.001	1.33 (1.17 to 1.51)	<0.001
Cardiovascular mortality						
Q1	1 (Reference)	<0.001†	1 (Reference)	0.005†	1 (Reference)	0.03†
Q2	1.86 (0.56 to 6.19)		1.91 (0.47 to 7.62)		1.59 (0.37 to 6.79)	
Q3	5.59 (1.92 to 16.27)		4.97 (1.41 to 17.46)		4.18 (1.14 to 15.25)	
Q4	5.67 (1.94 to 16.59)		6.57 (1.85 to 23.34)		4.93 (1.24 to 19.56)	
per 10^4 cells/μL	1.28 (1.14 to 1.43)	<0.001	1.37 (1.19 to 1.56)	<0.001	1.38 (1.17 to 1.63)	<0.001

Patients were quartiled according to reticulocyte count. Q1, $\leq 30.1 \times 10^3$ cells/μL; Q2, 30.2 to 39.4×10^3 cells/μL; Q3, 39.6 to 53.6×10^3 cells/μL; Q4, $\geq 53.7 \times 10^3$ cells. Model 1 is adjusted for age, sex, diabetic nephropathy, duration of dialysis, interdialysis weight gain, smoking status, vascular disease history, use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, systolic blood pressure, diastolic blood pressure, and weekly dose of recombinant human erythropoietin. Model 2 is adjusted for model 1 covariates and hemoglobin, white blood cell, platelet count, transferrin saturation, ferritin, phosphorus inorganic, creatinine, albumin, and C-reactive protein

Note: *HR* hazard ratio, *CI* confidence interval

†*p* for linear trend

for other risk factors known to affect mortality in HD patients, such as age, history of DMN, preexisting vascular disease, weekly dose of rHuEPO, Hb, WBC count, serum Alb, Cr, iP, or CRP. The correlation between all-cause mortality and reticulocyte count showed stronger statistical relation than the presence/absence of DMN, CRP, iP, and vascular disease history. Only age showed stronger independent association with outcomes. In addition, the strongest correlation was observed between cardiovascular mortality and reticulocyte count. There is only one previously reported study in the literature, by Cantaro et al., in which the prognostic value of the reticulocyte count was investigated [9]. This study, in which the data of 117 HD patients were analyzed for a period of 12 months and the reticulocyte count tended to be higher in the deceased group, however, failed to

Table 3 Correlation models for all-cause and cardiovascular mortality based on forward stepwise Cox proportional hazard regression

Variables	HR	95% CI	$\chi 2$	*p*
All-cause mortality				
Age (per 1 year)	1.08	1.06 to 1.11	40.00	<0.001
Reticulocyte count (per 10^4cells/μL)	1.36	1.22 to 1.51	29.85	<0.001
Diabetic nephropathy yes vs no	3.19	1.98 to 5.13	22.65	<0.001
C-reactive protein (per 1 mg/dL)	1.28	1.14 to 1.44	16.21	<0.001
Phosphorus inorganic (per 1 mg/dL)	1.24	1.03 to 1.46	5.18	0.02
Vascular disease yes vs no	1.77	1.06 to 2.95	4.84	0.03
Cardiovascular mortality				
Reticulocyte count (per 10^4 cells/μL)	1.40	1.23 to 1.60	25.38	<0.001
Diabetic nephropathy yes vs no	4.62	2.51 to 8.50	24.20	<0.001
C-reactive protein (per 1 mg/dL)	1.38	1.21 to 1.58	22.87	<0.001
Age (per 1 year)	1.08	1.04 to 1.10	21.15	<0.001
Diastolic blood presure (per 1 mmHg)	0.97	0.95 to 0.99	6.56	0.01

Stepwise, multivariate Cox proportional regression models were developed using candidate variables to predict all-cause and cardiovascular mortality. Candidate variables are age, sex, diabetic nephropathy, duration of dialysis, interdialysis weight gain, vascular disease history, use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, systolic blood pressure, diastolic blood pressure, weekly dose of recombinant human erythropoietin, hemoglobin, white blood cell, platelet count, transferrin saturation, ferritin, phosphorus inorganic, creatinine, albumin, C-reactive protein, and reticulocyte count. Inclusion criterion is $p < 0.05$

Note: *HR* hazard ratio, *CI* confidence interval

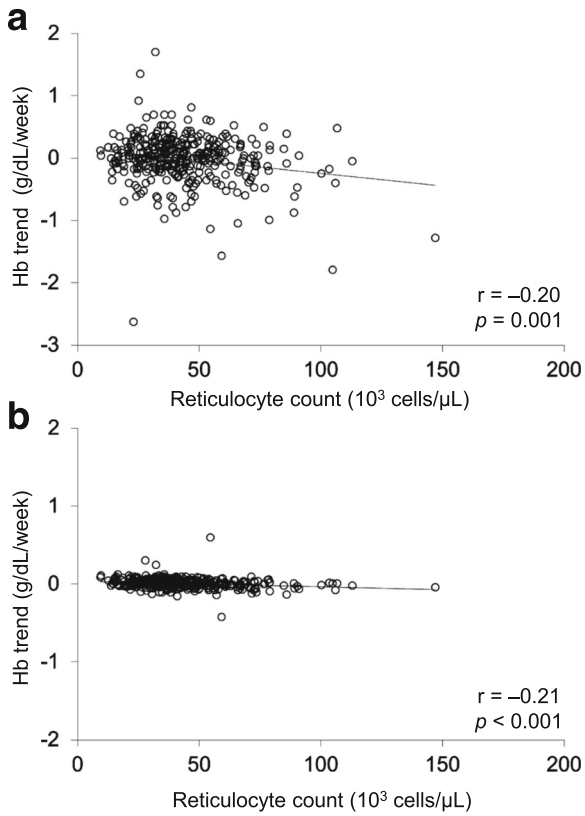

Fig. 2 Correlation of the reticulocyte counts with the Hb trends at 2 weeks and 6 months. To derive the temporal trend in hemoglobin, a best-fit (ordinary least squares) line of hemoglobin over time was plotted for each subject: the slope of this line represented the temporal trend of the hemoglobin level. **a** Correlation of the reticulocyte count with the Hb trend at 2 weeks ($n = 358$). The correlation coefficient was $r = -0.20$, $p < 0.001$. **b** Correlation of the reticulocyte count with the Hb trend at 6 months ($n = 338$). The correlation coefficient was $r = -0.21$, $p < 0.001$

show any significant correlation between the reticulocyte count and mortality. This discrepant result from our present study may be attributable to differences in the sample size, observation period, and/or methodology between the two studies.

The second important finding of this study was that reticulocyte counts showed a strong positive association with female gender, interdialytic body weight gain, and levels of serum EPO, WBC, LDH, iP, non-HDL, and glucose. EPO levels were assessed at intervals of more than 72 h from the last injection of rHuEPO; therefore, the impact of rHuEPO might have been small. Several studies have reported an increased level of plasma EPO in patients with heart failure, which was correlated to a more severe outcome [10–13]. Meanwhile, female gender, inflammation, hyperphosphatemia, hyperglycemia, and dyslipidemia were identified as potential risk factors of microvascular dysfunction [14–16]. In postmenopausal women, microvascular dysfunction has been linked with adverse outcomes, such as Syndrome X [14]. Recently, Shah et al. reported that global coronary flow reserve, which is defined as the ratio of stress to rest for absolute myocardial blood flow and is a reliable indicator of coronary microvascular function, may provide independent and incremental risk stratification for all-cause and cardiovascular mortality in patients with dialysis-dependent end-stage renal disease (ESRD) [17].

The third finding is that high reticulocyte counts were associated with negative Hb trends. The mean value of Hb at the time of enrollment was not significantly different among the four groups, and the slope of the change in Hb level over half of 1 year was very gentle (0.01 +/− 0.07 g/dL/w); however, the reticulocyte count was wide-ranging ($9.27 \times 10^3/\mu L$ to $146.9 \times 10^3/\mu L$). We theorize that those patients who need a high reticulocyte count to maintain the target Hb level may experience a poorer prognosis.

Given these findings, we would like to present two hypotheses for the relationship between high reticulocyte count and poor outcome. The first is that the reticulocyte count could be a sensitive marker of tissue hypoxia. Reductions in tissue oxygen tension due to microvascular dysfunction would be expected to lead to the activation of hypoxia-inducible transcription factors [15], which in turn might initiate the production of EPO. EPO increases reticulocyte release from the bone marrow and therefore prolongs the maturation time of circulating reticulocytes; this is likely the reason for findings showing a high reticulocyte count [18]. Even HD patients might produce EPO in response to chronic hypoxia. Indeed, Brookhart et al. suspected that ESRD patients who lived at high altitudes could increase endogenous EPO production [19].

The second hypothesis is that reticulocytosis is a result of the accelerated clearance of red blood cells (RBCs). A study showed that the RBC lifespan was significantly and negatively correlated with the erythropoiesis-stimulating agent (ESA) requirement of the patients [20]. Our findings imply that the reticulocytosis found in patients with a poorer prognosis may reflect the compensatory increase of erythropoiesis against the accelerated clearance of circulating RBCs as result of occult bleeding or impairment of RBC survival, including hemolysis [21], or increased eryptosis (apoptotic erythrocyte death) [22, 23]. Oxidative stress [24] and mechanical pressure [25] might play some roles.

There were some limitations to our study; first, it was a retrospective study without a pre-specified hypothesis; therefore, data on some important parameters were lacking. Although we speculated that hemolysis or eryptosis might have been the reason for the increased reticulocyte counts, we did not measure either the serum haptoglobin, phosphatidylserine-positive erythrocyte counts,

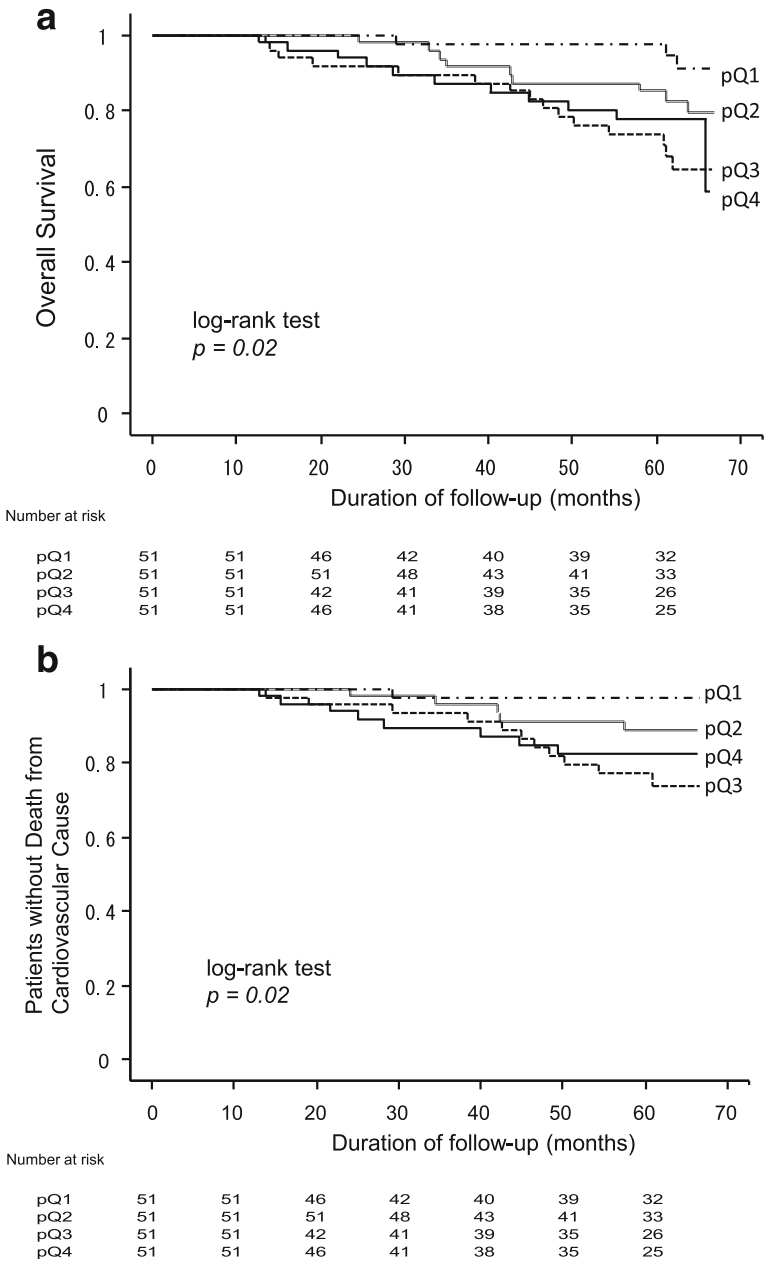

Fig. 3 Kaplan-Meier survival curves according to reticulocyte count quartiles at 1 year prior to enrollment. Patients were divided into quartiles according to their reticulocyte count: pQ1, ≤31.5×10^3 cells/μL; pQ2, 31.5×10^3 cells/μL to 42.1×10^3 cells/μL; pQ3, 42.2×10^3 cells/μL to 54.2×10^3 cells/μL; and pQ4, ≥54.4×10^3 cells/μL. **a** Overall survival; $p = 0.02$ as determined by a log-rank test conducted to compare the four groups. **b** Patients who had not died from cardiovascular causes; $p = 0.02$ according to a log-rank test conducted to compare the four groups

or spleen size. Second, our patients were treated with short-acting ESAs, and it would be unreasonable to extrapolate our findings to patients treated with darbepoetin or CERA. Finally, the average Hb level in our study subjects was lower than the target Hb level recommended in the guidelines published in Europe and the USA [26, 27], although this level was within the average range in Japan [28].

Conclusions

In summary, our findings demonstrated that higher reticulocyte counts in hemodialysis patients are associated with a higher mortality rate. The exact mechanisms underlying the association of the reticulocyte count with the mortality are still unknown. We propose that the increased reticulocyte count is a result of tissue hypoxia or is a compensatory mechanism to maintain

the target Hb concentration. Further studies are necessary to explore the mechanisms underlying the close association between the reticulocyte count and mortality in HD patients.

Abbreviations

ACE-I: Angiotensin-converting enzyme inhibitors; Alb: Albumin; ARB: Angiotensin receptor blockers; BMI: Body mass index; BUN: Blood urea nitrogen; CERA: Continuous erythropoietin receptor activator; CI: Confidence interval; Cr: Creatinine; CRP: C-reactive protein; DMN: Diabetic nephropathy; EPO: Erythropoietin; ESAs: Erythropoiesis-stimulating agents; ESRD: End-stage renal disease; Hb: Hemoglobin; HD: Hemodialysis; HR: Hazard ratio; iP: Phosphorus inorganic; LDH: Lactate dehydrogenase; non-HDL: Non-high-density lipoprotein; PLT: Platelet count; RBC: Red blood cell; rHuEPO: Recombinant human erythropoietin; SD: Standard deviation; TSAT: Transferrin saturation; WBC: White blood cell count

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Authors' contributions

CT conceived of the study. CT and KO participated in the design of the study, performed the statistical analysis, and drafted the manuscript. HM took the lead in the data collection. NN and YN participated in its design and edited the manuscript. All authors read and approved the final manuscript.

Competing interests

N.N. is a member of the speaker's bureau for Kyowa Hakko Kirin Co., Ltd. The authors declare that they have no competing interests.

Consent for publication

A preliminary simplified version of this manuscript was already published in Japanese book in Japanese (Hemodialysis Treatment NEXT XIV: pp. 84–90, 2012). In this time, we have changed the analysis method and thus the present figures and tables are different from the former published ones. The authors will transfer copyright to the publisher upon acceptance of the manuscript.

Ethics approval and consent to participate

This is a retrospective observational study. All data analyzed were collected as part of routine diagnosis and treatment. Patients had been treated according to Japanese Society for Dialysis Therapy guidelines. The ethics committee in our hospital judged that an ethical clearance was unnecessary for a retrospective data analysis. Hence, we did not obtain an ethics committee's approval for this study.

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